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Frontal brain deactivation during a non-verbal cognitive judgement bias test in sheep

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Animal welfare concerns have raised an interest in animal affective states. These states also play an important role in the proximate control of behaviour. Due to their potential to modulate short-term emotional reactions, one specific focus is on long-term affective states, that is, mood. These states can be assessed by using non-verbal cognitive judgement bias paradigms. Here, we conducted a spatial variant of such a test on 24 focal animals that were kept under either unpredictable, stimulus-poor or predictable, stimulus-rich housing conditions to induce differential mood states. Based on functional near-infrared spectroscopy, we measured haemodynamic frontal brain reactions during 10 seconds in which the sheep could observe the configuration of the cognitive judgement bias trial before indicating their assessment based on the go/no-go reaction. We used (generalised) mixed-effects models to evaluate the data. Sheep from the unpredictable, stimulus-poor housing conditions took longer and were less likely to reach the learning criterion and reacted slightly more optimistically in the cognitive judgement bias test than sheep from the predictable, stimulus-rich housing conditions. A frontal cortical increase in deoxy-haemoglobin [HHb] and a decrease in oxy-haemoglobin [O_2Hb] were observed during the visual assessment of the test situation by the sheep, indicating a frontal cortical brain deactivation. This deactivation was more pronounced with the negativity of the test situation, which was reflected by the provenance of the sheep from the unpredictable, stimulus-poor housing conditions, the proximity of the cue to the negatively reinforced cue location, or the absence of a go reaction in the trial. It seems that (1) sheep from the unpredictable, stimulus-poor in comparison to sheep from the predictable, stimulus-rich housing conditions dealt less easily with the test conditions rich in stimuli, that (2) long-term housing conditions seemingly did not influence mood—which may be related to the difficulty of tracking a constant long-term state in the brain—and that (3) visual assessment of an emotional stimulus leads to frontal brain deactivation in sheep, specifically if that stimulus is negative.

Keywords: affective states, cognitive judgment bias test, fNIRS, frontal brain, sheep

1. Introduction

Along with researchers' interest in animal welfare, the number of studies on animal affective states has recently increased (e.g. Boissy et al., 2007; Mendl et al., 2010; Paul et al., 2005). Long-term affective states, that is, mood, are of specific interest because of their potential far-reaching consequences; negative mood could taint all emotional experiences as seen in clinical depression in humans (Grippo and Johnson, 2009; Groenewold et al., 2013) or, alternatively, positive mood could allow animals to more easily cope with short-term negative experiences (Laeger et al., 2012; Reefmann et al., 2012). Also, mood is likely to play a fundamental role as part of the proximate control mechanisms of behaviour because it modulates reactions in response to emotional stimuli, for example, by influencing the cognitive assessment of such stimuli (Mendl et al., 2010). To tap mood in non-verbal subjects, such as small children or animals, a non-verbal cognitive judgement bias paradigm has been proposed and repeatedly implemented (Gygax, 2014; Mendl et al., 2009). In this paradigm, animals are trained with cues predicting more positive or more negative consequences and are then tested with additional ambiguous cues. Their reaction to the ambiguous cues reflects whether they assess the situation to be more similar to the negative cue, that is, pessimistically (with a negative cognitive judgement bias), or more similar to the positive cue, that is, optimistically (with a positive cognitive judgement bias).

In animals, mood as reflected in alterations of their reactions to at least some of the ambiguous cues has successfully been altered by introducing unpredictable events in housing and management before subjecting the animals to a cognitive judgement bias test (e.g. Destrez et al., 2013; Doyle et al., 2011; Harding et al., 2004) and by decreasing or increasing stimulus richness of the housing environment (e.g. Bateson and Matheson, 2007; Burman et al., 2008; Douglas et al., 2012).

In some studies, additional measurements were taken during the trials of the cognitive judgement bias test to better understand and more easily interpret the animals' reactions. In rats, Richter et al. (2012) observed head dips, rearing-up on the walls and entries to the arms

of a radial maze type of cognitive judgement bias set-up. They found that negative arms were visited more often than positive arms and that rats performed more head dips in the negative arm. Verbeek et al. (2014) observed behaviour of sheep during their choices in a spatial cognitive judgement bias paradigm but did not find differences in the numbers of steps or vocalisations or the duration of oral manipulations of the environment. Finally, Düpjan et al. (2013) collected salivary cortisol samples before and after test sessions in a spatial cognitive judgement bias paradigm but did not find a significant influence of the experimental conditions.

Given that the frontal cortex in humans is heavily involved in cognitive assessment of situations (e.g. Ray and Zald, 2012) and in the assessment of the valence of stimuli, that is, their negativity or positivity (e.g. Berridge and Kringelbach, 2013; Etkin et al., 2011), measurements of the activation of the frontal cortex in the context of a cognitive judgement bias test provide the potential to obtain valuable information on the brain processes involved in the assessment and decision making in the cognitive judgement bias test. Functional near-infrared spectroscopy reflecting haemodynamic changes in the brain allows for such measurements in freely moving animals in a non-invasive way (e.g. Muehlemann et al., 2011; Vögeli et al., 2014).

In the current experiment, we wanted to investigate whether the valence of the cue in a cognitive judgement bias test was reflected in the frontal cortical activation in sheep and whether this reaction was modified by mood. Therefore, we kept sheep under either unpredictable, stimulus-poor or predictable, stimulus-rich housing conditions to induce differential mood states, conducted a spatial cognitive judgement bias test and measured frontal cortical brain reactions when the sheep could visually assess the configuration of each trial before entering the test arena. We expected that sheep from the unpredictable, stimulus-poor housing conditions would have a low probability of approaching ambiguous locations and would therefore show a negative judgement bias. Furthermore, we expected that frontal cortical brain activation would increase with proximity of the cue location towards the negative cue (Gygax et al., 2013; Vögeli et al., 2014) or with sheep showing a no-go

reaction in the trial and that the brain activation of the sheep from the unpredictable, stimulus-poor housing conditions would be stronger than those of the sheep from the predictable, stimulus-rich housing conditions (as seen in behaviour by Reefmann et al., 2012).

2. Material and methods

This project was assessed by the Swiss National Science Foundation, and all procedures have been approved by the Cantonal authority (Canton of Thurgau, permits nos. F6/10 and F4/11 for conducting animal experiments).

2.1 Animals and housing groups

Twenty-nine female non-lactating and non-gestating Lacaune sheep about 2.5 years of age made up the two groups used in this experiment (Vögeli et al., 2014). 24 focal sheep (12 from each housing group) that had previously been chosen at random were included in the cognitive judgement bias test. With one exception, these focal sheep had been involved in a series of experiments in which they were exposed to different sets of stimuli thought to induce emotional reactions (Vögeli et al., 2014, submitted) and in a previous cognitive judgement bias test (Vögeli et al., 2014). However, the one replacement sheep did not reach the test phase in the current experiment.

To induce a relatively more negative or more positive mood, the sheep had been subjected to either unpredictable, stimulus-poor or predictable, stimulus-rich housing conditions, respectively, since July 2011 (Vögeli et al., 2014). To induce a negative mood, a group of 14 sheep was continuously housed in an enclosed deep-litter pen where feed, water and daylight were provided daily at unpredictable times. However, after the sheep were housed in these conditions for several months, no large difference in respect to the sheep's reactions in a cognitive judgement bias setting was found (Vögeli et al., 2014). To increase the contrast between the two housing conditions, the unpredictable, stimulus-poor conditions were first relaxed and later tightened again, such that the cognitive judgement bias test in the current

144 experiment could be expected to pick up this recent deterioration in housing conditions in
145 addition to the long-term conditions per se. During the time when conditions were relaxed
146 (starting end of April 2013), sheep in the unpredictable, stimulus-poor housing conditions
147 temporarily had access to extra space outside their enclosed pen and thus experienced the
148 natural daylight rhythm and additional visual stimuli from outside their pen. When conditions
149 were tightened (from the beginning of June 2013 onwards), sheep were kept in the
150 unpredictable, stimulus-poor conditions described above. In addition, the pen was split into
151 two compartments with two subgroups of seven sheep each. Every 2 to 5 days, three
152 randomly chosen sheep were exchanged between the two subgroups to mimic social
153 instability. In contrast to the initial set of conditions, one of the subgroups now received feed
154 at regular feeding times whereas the other subgroup needed to wait a random amount of
155 time up to 2 hours until feed was provided. Regular and random feeding times were
156 alternated between the two subgroups. Water was available ad libitum in both subgroups.

157 To induce a positive mood, 15 sheep were housed in a generous open-front pen with access
158 to an exercise yard on work days between 08:15 and 17:30 h providing a natural daylight
159 rhythm. At night, these sheep were on pasture. Furthermore, sheep were fed twice a day at
160 regular times between 07:30 and 08:00 h and between 16:30 and 17:00 h. All sheep had
161 permanent access to water.

162

163 2.2 Cognitive judgement bias test

164 Sheep took part in an experiment in which they were confronted with thermal stimuli (Vögeli
165 et al., unpublished) in July and August 2013 after which housing conditions were again left
166 undisturbed for 2 weeks. We then conducted a spatial cognitive judgement bias test between
167 mid-September and early November 2013 with a positively and a negatively reinforced cue
168 box in the corners of a test arena and three unreinforced ambiguous cue boxes at locations
169 between the corners (Vögeli et al., 2014). Cue boxes opened automatically when sheep
170 approached to 1.1 m (Fig. 1, left; Vögeli et al., 2014), and in any one trial, only one cue was

171 presented. The positively reinforced box contained a reward consisting of concentrated feed
172 and salt. The negatively reinforced box aimed at having a frustrating or punishing effect on
173 the animals and therefore contained straw that was coloured pink as well as a green LED
174 point light source. The content of the negatively reinforced box was chosen to elicit more
175 visits than a more aversive stimulus used in a previous study (Vögeli et al., 2014). At the
176 ambiguous locations, the cue box was empty which could reduce the motivation of the sheep
177 to approach the ambiguous cues after an initial visit to such a cue without consequence.
178 Here, this effect should be small because each ambiguous cue was presented to each sheep
179 only once. Also, a re-evaluation of the data from Vögeli et al. (2014) did not indicate a
180 consistent reduction in approach behaviour, once the sheep had visited an unrewarded
181 ambiguous cue.

182 The test arena included a waiting area (3.4 m x 2.0 m) and a choice area (3.4 m x 4.7 m) and
183 was enclosed by an opaque fence (1.9 m high). A wooden wall (1.5 m high) separated the
184 waiting and the choice areas. A two-part sliding door was placed in the middle of the wall. In
185 each trial, a window (width x height : 76 x 61 cm) allowing visual assessment of the choice
186 area was provided by sliding away the opaque part of the door and sliding the part with the
187 window into place (Fig. 1, left). Ten seconds after a sheep directed its head towards the
188 window, it was allowed access to the choice area for 90 seconds. In these 90 seconds the
189 door between the waiting and the choice area remained open but sheep were coaxed to
190 enter the choice area by calmly touching the sheep on the back if they did not enter on their
191 own. The test was composed of two training phases and a subsequent testing phase. Each
192 sheep received a maximum of one session with five trials per day and was always trained
193 and tested at about the same time of day.

194 In the first training phase, sheep were separated from the group and individually guided to
195 the waiting area. The positively reinforced box was positioned in one of the two opposite
196 corners of the choice area. The side (left/right) of the positive cue was balanced across
197 sheep. For each sheep, the same side was used as in the previous cognitive judgement bias
198 test (Vögeli et al., 2014). Sheep were then lured and accompanied to the positively

reinforced box by the experimenter until they independently approached the box. When sheep opened this box in the 15 trials of three consecutive sessions, they had reached the first learning criterion and moved to the second training phase.

In the second training phase, the sheep were additionally confronted with the negatively reinforced box, which was positioned in the corner opposite to the positively reinforced box. The different cue positions were presented one at a time with three positively and two negatively reinforced box positions per session. No box position occurred in more than two consecutive trials, and each session ended with the positively reinforced box. At least one opening of the negatively reinforced box was provoked with each sheep. When sheep did not approach the positively reinforced box at all, they were again lured to the box by the experimenter. When sheep opened the positively reinforced box and avoided the negatively reinforced box in the 15 trials of three consecutive sessions, they reached the final learning criterion and moved on to the test phase. This criterion reflects a non-random choice in three successive sessions as based on a one-sided binomial distribution with $p = 0.5$. A maximum of 36 training sessions were run in the two training phases together.

In the test phase, sheep went through one session with five trials. In addition to the negatively and positively reinforced box locations in each corner of the testing area, three ambiguous locations were presented. The ambiguous box positions were at a relative distance of 30% (at 0.9 m), 50% (at 1.5 m) and 70% (at 2.1 m) between the positions of the positively and the negatively reinforced boxes. The sequence of the box locations was chosen at random, and each sheep was tested with a different sequence. Opening the box was considered a go response whereas not opening the box within 90 seconds was considered a no-go response.

In addition to the go/no-go response, we measured frontal cortical brain activation by using functional near-infrared spectroscopy (fNIRS; Muehlemann et al., 2008, 2011; Vögeli et al., 2014) during the 10 seconds when the sheep could visually assess the choice area. We chose this period before the animals performed their actual choice in order to control for the

current behaviour, that is, all sheep during all the measurements were looking through the window of the sliding door into the choice area. Therefore, any difference found in frontal brain activation was caused by what the sheep saw in the choice area and not by the behaviour that followed. Based on the raw absorption data, we calculated relative changes in oxy- [O_2Hb] and deoxy- [HHb] haemoglobin concentrations at 1 Hz during a total of 30 seconds (10 seconds pre-assessment phase, 10 second of visual assessment, 10 seconds post-assessment phase). All animals were habituated to wearing the measurement equipment eight times during an experiment conducted in July and August 2013 (Vögeli et al., unpublished) and another three times during the training for the present cognitive judgement bias test. For the cognitive judgement bias test, the sheep were equipped with the fNIRS device (Fig. 1 right) immediately when brought to the waiting area. They were then left undisturbed for 2 min before the window to the choice area was opened.

2.3 Statistics

R version 3.1.0 (R Core Team, 2014) was used for all statistical analyses. Model selection was performed by using model probabilities (weights) based on the Bayesian information criterion (BIC; Gygax et al., 2013) using an all-subset strategy in modelling the go/no-go response (function dredge in package MuMIn; Barton, 2014) and a specific set of models in evaluating the fNIRS reaction (modified function aictab in package AICcmodavg; Mazerolle, 2013). Model assumptions were checked by graphical analysis of the residuals.

To analyse which boxes were opened during the cognitive bias test, a generalised mixed-effects model based on the binomial distribution was used (function glmer in package lme4; Bates et al., 2014) by following the strategy suggested in Gygax (2014). The possible fixed effects included the housing condition (factor with two levels: predictable, stimulus-rich; unpredictable, stimulus-poor), the position of the box (continuous as the proportion of the distance from the negative location) and their interaction. The random effect was the animal identity.

For the analysis of the fNIRS data, we used linear mixed-effects models (function lme in package nlme; Pinheiro et al., 2014). Changes in [O_2Hb] and [HHb] were transformed according to Gygax et al. (2013) to satisfy statistical assumptions, and an auto-regressive process of order 3 was included for the residuals (ibidem). Due to artefacts, two single light paths for one animal were excluded. One stimulus for one additional animal was missing because of technical failure of the measurement device. The random effects were the light path nested within trial and sheep identity.

The minimum model in the analysed set was the null model, and the maximum model contained the fixed effects housing condition (level with two factors: unpredictable, stimulus-poor; predictable, stimulus-rich), position of the box (continuous relative position), whether or not a go response followed the visual assessment (factor with two levels: no-go and go reaction), the time course throughout the stimulation (a spline based on time coded as a continuous variable), location of the measurement paths on the head (lateral position: indicator for left versus right hemisphere; longitudinal position: indicator for cranial versus caudal location; measurement depth: deep versus superficial measurement) and all their possible interactions.

First, the degrees of freedom for the spline were selected among the numbers 5, 9 and 13 based on the full model. The number of degrees of freedom influences the amount of curvature possible in the model estimates. This number was taken as fixed for the model selection in respect to the fixed effects. The null and the full models (two models) were complemented by a set of models that all included either the time course only (one model) or interactions with the time course (15 models) implying that the other explanatory variables (i.e. the housing group, the position of the box, whether a go response followed, or the measurement location on the head) would modify the time course. In addition, all models that included valence and that were not over-specified were run with position of the box coded as a factor with five levels reflecting potential non-linear effects of the position of the box (six models). The complete set of models analysed therefore included 24 models.

3. Results and Discussion

3.1 Behaviour during training and the cognitive judgement bias test

The sheep from the predictable, stimulus-rich housing conditions reached the final learning criterion faster than the sheep from the unpredictable, stimulus-poor conditions, that is, in a median of 17 versus 37 sessions (Mann-Whitney-U test: $W = 112.5$, $p = 0.02$), respectively, and they were more likely to do so (12/12 versus 5/12 sheep, respectively; Fishers-exact test: $p = 0.005$). This difference in learning speed was largely based on the number of sessions that the sheep needed for the first part of the training in which they were supposed to approach the positive cue box. Once they reached the second phase of the training, the sheep that did learn reached the second criterion in a maximum of 7 sessions with 11 of the 17 sheep doing so in 4 sessions. The probability of a sheep's go response increased with the increasing distance from the negative box position (model including the box position: model probability, $mPr = 0.85$, $E_0 > 1700$). In addition, the go response was modulated by housing group (model including the main effects box position and housing condition: $mPr = 0.12$, $E_0 > 246$; Fig. 2a in Gygax, 2014). This difference indicated that the probability of a go reaction, that is, opening a box, was lower for sheep from the predictable, stimulus-rich conditions compared with the sheep from the unpredictable, stimulus-poor conditions. All other models reached model probabilities < 0.03.

Compared with sheep from the predictable, stimulus-rich environment, sheep kept in the unpredictable, stimulus-poor housing conditions were less likely and slower in reaching the learning criterion of the cognitive judgement bias test. This result agrees with that from the prior test conducted with the same animals (Vögeli et al., 2014). As discussed there, this difference would be consistent with the notion that the sheep in the unpredictable, stimulus-poor housing conditions were in a more negative mood than the sheep in the predictable, stimulus-rich housing conditions, though it is possible that the sheep from the unpredictable, stimulus-poor conditions—in contrast to those from the predictable, stimulus-rich

conditions—were more challenged by the richness of stimuli with which they were confronted during the training for the cognitive judgement bias test. The two housing conditions differed by several other aspects as well, such as lighting conditions (i.e., photoperiodism), overall space, density of the animals in the pen, and the available quality of the ground. It cannot be excluded that these aspects also had an effect on the performance of the sheep. Finally, it may be noteworthy that the sheep needed a similar number of trials to reach the learning criterion in the current test as in the test conducted approximately one year earlier (Vögeli et al., 2014). This observation is in contrast to the recent finding that tested goats remembered a complex two-step foraging task well after 10 months (Briefer et al., 2014).

No-go responses in a go/no-go paradigm are not easily interpreted because they might reflect an omission of a reaction rather than an active choice. In our case, the sheep usually stayed at the end of the choice area far from the cue box or even turned around and went back to the waiting area. This behaviour seemed to indicate an active avoidance of the cue box during the no-go reaction. We found only a small difference in the probability of a go response between the two housing conditions and thus little evidence for mood differences although other studies that manipulated predictability (e.g. Destrez et al., 2013; Doyle et al., 2011; Harding et al., 2004) and stimulus richness (e.g. Bateson and Matheson, 2007; Burman et al., 2008; Douglas et al., 2012) reported significant differences. If at all, the sheep from the predictable, stimulus-rich conditions made more pessimistic choices than the sheep from the unpredictable, stimulus-poor conditions. Some authors have suggested that such a result could be explained as a release from a negative situation that would lead to a more optimistic judgement bias (e.g. Burman et al., 2011; Doyle et al., 2010). As our conditions were not acute, this explanation seems unlikely. Alternatively, animals in general and our sheep in particular might find it difficult to perceive and monitor constant long-term conditions in contrast to the ease with which changes in conditions are perceived (e.g. Rolls, 2014). This could mean that the sheep habituated even to the unpredictable, stimulus-poor conditions. Also, during long periods with constant conditions, random fluctuations in conditions or random events might overshadow the actual conditions (Vögeli et al., 2014).

335 The relatively long training period and strict learning criterion could have resulted in small
336 differences between the two housing groups, too, either when animals were frustrated by the
337 long succession of training sessions or if training provided an enrichment for the sheep
338 (Melfi, 2013; Westlund, 2014). Finally, the small difference between the sheep from the two
339 housing conditions could result from selection bias in that only the most resilient sheep from
340 the unpredictable, stimulus-poor housing conditions reached the learning criterion and were
341 included in the test whereas larger differences would have been found if all sheep could have
342 been tested.

343

344 3.2 Functional near-infrared spectroscopy (fNIRS)

345 A low number of degrees of freedom (df = 5) in the splines modelling the time course was
346 adequate for both outcome variables, [O_2Hb] and [HHb] (both *mPr* = 1.00). The most likely
347 models for [O_2Hb] and [HHb] included the time course only (Table 1) indicating that [O_2Hb]
348 decreased and [HHb] increased as long as the sheep could visually assess the choice area
349 with the configuration of the trial they were about to enter (Fig. 2).

350 There was some indication that the decrease in [O_2Hb] was stronger in the sheep from the
351 unpredictable, stimulus-poor compared with the sheep from the predictable, stimulus-rich
352 conditions (Table 1; Fig. 2). There was an alternative, even weaker indication that the
353 decrease in [O_2Hb] was less pronounced with increasing distance from the negatively
354 reinforced box or when a go response followed (Table 1; Fig. 2). The same three alternative
355 models were the next most probable models for [HHb], but they reached only a very small
356 absolute model probability (Table 1), and accordingly model estimates differed little from the
357 model including the time course only (Fig. 2).

358 Our measurements of frontal brain activation by using fNIRS seemed to indicate frontal brain
359 processes specific to the visual assessment of the choice area the sheep were about to enter
360 in the cognitive judgement bias test. Contrary to our expectations, the [HHb] increase and
361 [O_2Hb] decrease during the assessment indicated a general frontal brain deactivation during

362 the visual assessment of the cue boxes. This deactivation was more pronounced when the
363 situation was more severe, reflected by the sheep's provenance from the unpredictable,
364 stimulus-poor housing condition, the spatial proximity to the negatively reinforced cue, or the
365 no-go reaction in the trial. Whereas the distance from the negative cue position and the go
366 response were partly correlated—because more sheep would approach the cue boxes that
367 were farther away from the negatively reinforced position, specifically the positively
368 reinforced box—these two variables did not correlate with the provenance from the two
369 housing conditions. Though all these effects were rather weak and could not be well
370 distinguished statistically, they coincide with our previous findings of a frontal brain
371 deactivation when sheep were confronted with video images of intraspecific agonistic
372 interactions which were presumed to be negative (Vögeli et al., submitted; see also Goldberg
373 et al., 2006). This deactivation contrasts the activation found in frontal cortical brain areas
374 observed in response to non-visual negative stimuli in goats (Gygax et al., 2013) and sheep
375 (Vögeli et al., 2014). It also contrasts the more general findings on the role of the frontal
376 cortical areas in decision making in humans, non-human primates, and rodents (e.g. Kesner
377 and Churchwell, 2011; Dixon and Christoff, 2014) suggesting that the involved frontal brain
378 areas become more active during decision making.

379

380 In conclusion, it seems that (1) sheep from the unpredictable, stimulus-poor in comparison
381 with sheep from the predictable, stimulus-rich housing conditions dealt less easily with the
382 test conditions rich in stimuli as seen in the number of trials needed to reach the learning
383 criterion and the likelihood that they reached this criterion, that (2) long-term housing
384 conditions seemingly did not influence mood, which may be related to the difficulty of
385 monitoring a constant long-term state, and that (3) visual assessment of an emotional
386 stimulus leads to frontal brain deactivation in sheep, specifically when that stimulus is
387 negative.

388

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Table 1

Model structure, delta Bayesian information criterion (BIC) in comparison to the most likely model, model probability, and evidence ratio when compared with the null model (E_0) for all models discussed in the text.

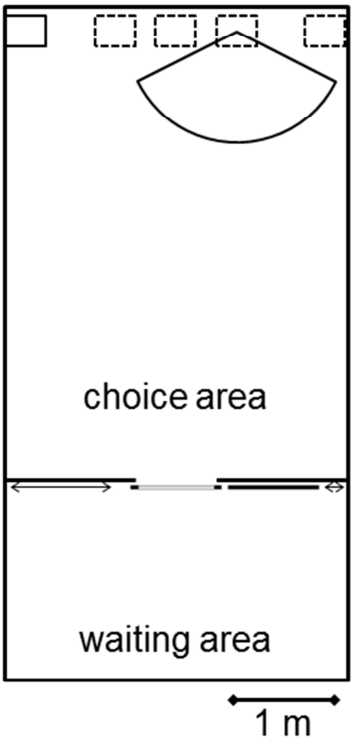
Model structure[a]	Delta BIC	Model probability	E_0
[HHb]			
Time	0.00	1.00	2.28×10^{25}
Time * box position	37.30	7.94×10^{-9}	1.81×10^{17}
Time * housing condition	37.37	7.68×10^{-9}	1.75×10^{17}
Time * go/no-go reaction	41.51	9.70×10^{-10}	2.21×10^{16}
Other models in the set	>104.45	$<1^{-10}$	<475.97
Null model	116.78	$<1^{-10}$	1
[O_2Hb]			
Time	0.00	0.901	7.56×10^{61}
Time * box position	23.92	5.75×10^{-6}	4.83×10^{56}
Time * housing condition	4.43	0.099	8.27×10^{60}
Time * go/no-go reaction	20.34	3.45×10^{-5}	2.90×10^{57}
Other models in the set	>81.24	$<1^{-10}$	$<1.73 \times 10^{44}$
Null model	284.96	$<1^{-10}$	1

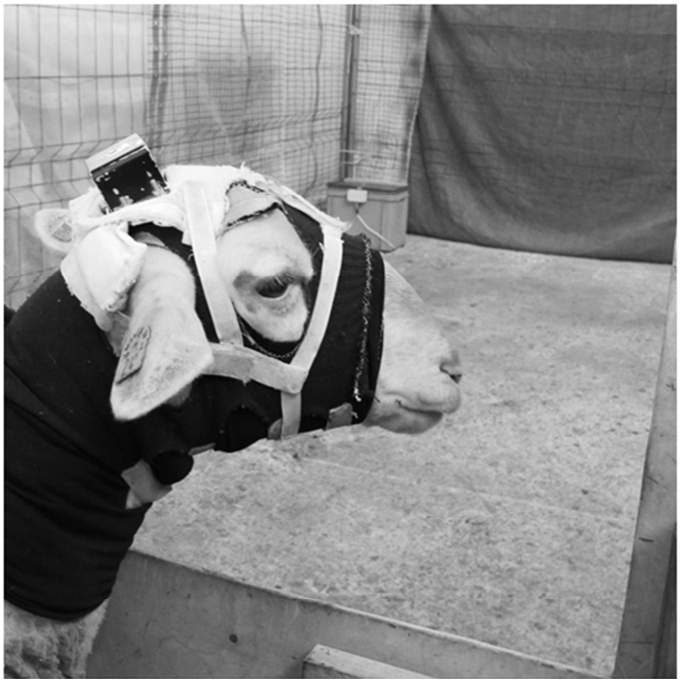
[a]Fixed effects as described in the methods section: time as a natural spline with five degrees of freedom; *: indicates the inclusion of an interaction between the two variables listed.

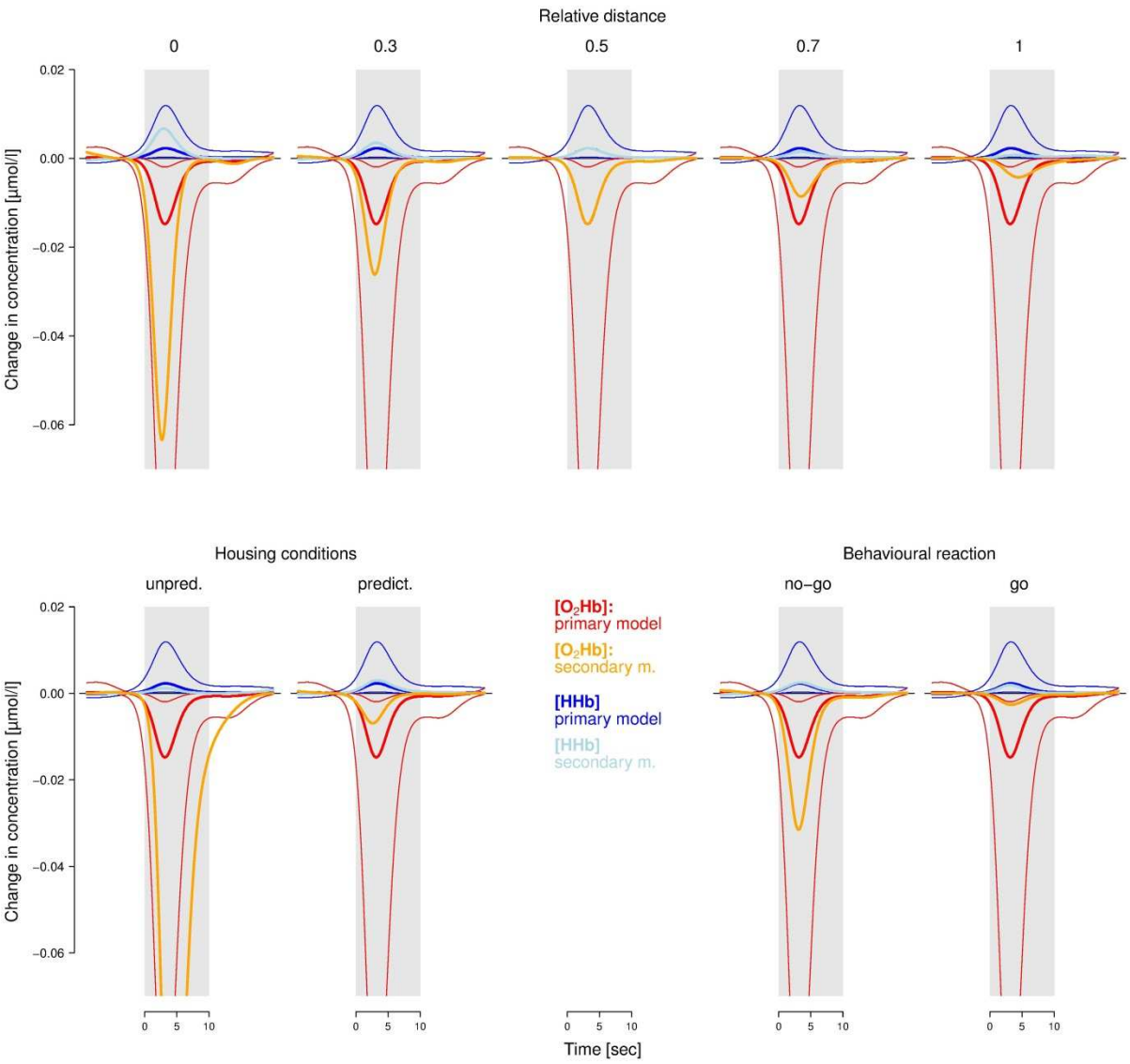
Figure Legend:

Figure 1. Left: Schematic view of the test arena with locations of the cue boxes (only one cue box was present at any one time). The part-circle at the right-hand ambiguous position indicates the approach distance at which the cue box opened automatically. The grey part in one of the sliding doors indicates the position of the window to the choice area, and arrows indicate how the sliding doors could be moved. Right: Sheep equipped with fNIRS measurement device at the window to the choice area with one cue box in the background.

Figure 2. Average relative haemodynamic changes during the period when sheep could visually observe the choice area of a cognitive judgement bias test at the start of a given trial (grey bars). Thin lines indicate 95% confidence intervals of the primary models (models identical in all subfigures). Average changes in a set of secondary models are presented in relation to the relative distance of the box positions, the housing conditions of the sheep (unpredictable, stimulus-poor and predictable, stimulus-rich) and whether the sheep approached the box after looking at it.


cue
box
choice area
waiting area
1 m




Relative distance
0
0.3
0.5
0.7
1
Change in concentration [µmol/l]
0.02
0.00
−0.02
−0.04
−0.06
Housing conditions
unpred.
predict.
Behavioural reaction
no−go
go
[O2Hb]:
primary model
[O2Hb]:
secondary m.
[HHb]
primary model
[HHb]
secondary m.
0
5
10
Time [sec]